NEW DIETARY INGREDIENT NOTIFICATION FOR lactium™

In accordance with the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 U.S.C. §350b (a) (2), and with final regulations published in the Federal Register (1997, 62:49886-49892, 21 C.F.R. § 190.6) "Requirement for Premarket Notification", the following information is submitted by Ingredia in support of a New Dietary Ingredient Notification for lactium™. Ingredia intends to market lactium™ as a New Dietary Ingredient for a dietary supplement in the United States. As per the statutes of the DSHEA, 21 U.S.C. § 350b (a) (2), Ingredia will not introduce, market, distribute or sell lactium™ until at least 75 days following official acknowledgement of the receipt of this notification by the U.S. Food and Drug Agency (FDA).

SECTION 1

The name and complete address of the manufacturer of the dietary ingredient.

The manufacturer of the dietary ingredient will be:

Ingredia
Site de Saint Pol s/ Ternoise
ZI route d'Ostreville
62130 Saint Pol sur Ternoise
France

Contact: Jean-François Boudier
Scientific Director,
Ingredia
51-53 Avenue F. Lobbedez, B.P 946
62003 ARRAS Cedex
France
Tel. 33-(3)-21-23-80-36
Jf.boudier@ingredia.com

The importer and distributor of the new dietary ingredient will be:

ADVITECH Solutions.
1165, boulevard Labourneuf
Suite 310
G2K 2C9 VANIER
QUEBEC
Canada

SECTION 2

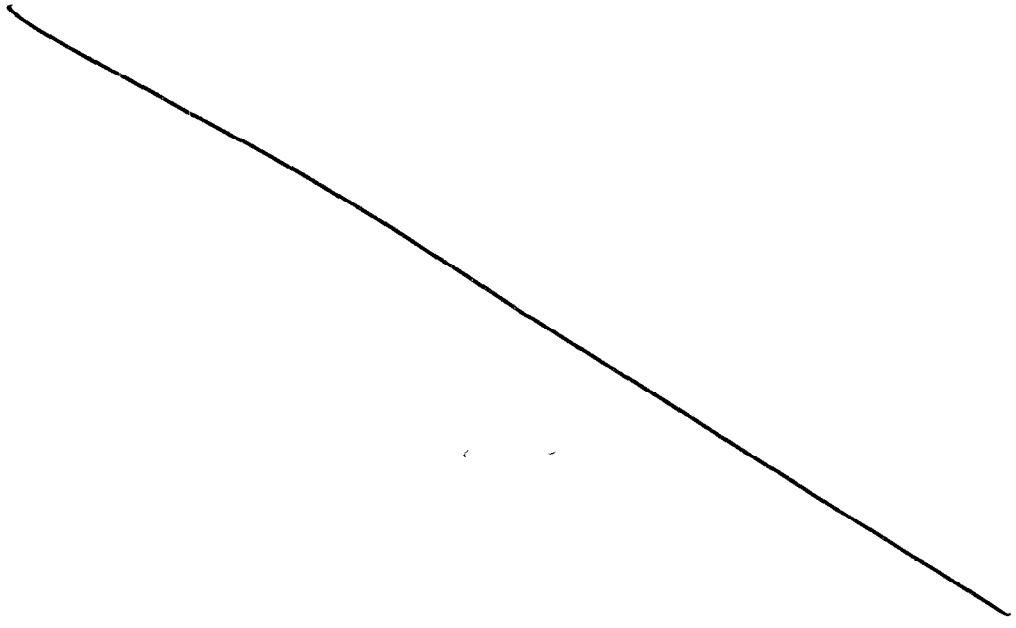
The name of the dietary ingredient.

The new dietary ingredient is lactium™ . It is a peptone that contains the bioactive decapeptide α_{s1} casein (f91-100) (also called α-casozepine). lactium™ originates from bovine skimmed milk, and during its manufacture, α_{s1} casein enriched fraction is separated from the skimmed milk and is subsequently hydrolysed by the proteolytic enzyme trypsin, which s a protease found in the gastrointestinal tract of the newborn and adults in equal concentrations. Overall, the hydrolysis reaction would be identical to a process which occurs naturally in the human gut. The degree of hydrolysis (DH) is less than 20%; thus, the resulting lactium™ product is a casein preparation of polypeptides, oligopeptides, and amino acids. The bioactive decapeptide α_{s1} casein (f91-100) has been isolated and identified using chromatographic techniques and binding assays. The minimum content of the bioactive decapeptide (molecular mass = 1266.1 Da), in lactium™ is 1.8%.

The hydrolysis is quite identical to a step of the process which naturally occurs in the gut.

See *appendices 1 and 2* for specifications and analysis certificates.

Manufacturing Information



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SECTION 3

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labelling of the dietary supplement, or if no conditions of use are recommended or suggested in the labelling of the dietary supplement, the ordinary conditions of use of the supplement.

lactium™ will be marketed for use in products meeting the definition of "dietary supplement" in section 201 (ff) of the Federal Food, Drug, and Cosmetic Act. lactium™ will be sold and incorporated in the form of oral serving (*e.g.*, pills, tablets, gums) containing approximately 150 to 400 mg per capsule. Consumption of 1 oral serving per day will be suggested or recommended. Based on consumption of 1 oral serving per day, the maximum amount of lactium™ that a consumer will receive is 400 mg/day, which is the highest indicated quantity of lactium™ that will be used in the dietary supplement, or approximately 6.67 mg/kg body weight/day for a 60 kg person. The principle display label would also indicate that the product is manufactured from α_{s1} casein and that the product is recommended for the use of adults only.

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labelling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

The overall safety of lactium™ is supported by the results of toxicology testing in animals, and clinical studies in humans. The toxicology of lactium™ has been investigated in oral studies in rats including an acute single dose study and a repeated dose study of 4 weeks in duration. In addition, reproductive toxicity and mutagenicity studies were performed that examined the potential for fertility, or developmental effects in rats, and the potential for mutagenic activity in L5178Y mouse lymphoma cells respectively. A 30-day clinical trial was also conducted in normal, healthy volunteers. The Generally Recognized As Safe (GRAS) status of peptones as well as the historical exposure of hydrolyzed alpha s1 casein from infant formula and bovine milk also provides supporting evidence that use of lactium™ as a dietary supplement would be safe.

Animal Studies

(i) Acute Single Dose Toxicity

An acute single dose toxicity study of lactium™ was conducted in female Sprague-Dawley rats (Gomond and Miermon, 2003) *(appendix 3)*. The study was conducted in accordance with Good Laboratory Practices (GLP), and the protocol was based on the Organisation for Economic Co-operation and Development (OECD) guideline 423 of December 17, 2001, Annex 2 d. Animals (6 nulliparous and non-pregnant females) were orally administered 2,000 mg lactium™/kg body weight diluted with distilled water. The study evaluated body weight the day before administration, then on Days 1, 4, 8, and 15, clinical and behavioural signs, necropsy findings and mortality, expressed in percentage of compound-related deaths. All animals were sacrificed on Day 15 and necropsied. This study was performed in duplicate.

There were no changes in body weight, clinical or behavioural changes, and no negative findings upon necropsy. According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, lactium™ was classified in the hazard category of 5 or unclassified (OECD, 2001), with an LD_{50} greater then 2000 mg/kg in the rat. *(appendix 4)*.

(ii) Repeated Dose Toxicity

Dufour *et al.*, (2003) *(appendix 5)*. conducted a 28-day oral repeated dose (gavage) toxicity study of lactium™ in male and female Sprague-Dawley rats. The study was conducted in accordance with GLP and the protocol was based on OECD guideline 407 of July 27, 1995 and Appendix IV.D part B7 of the European Directive 96/54/EEC of July 30, 1996. Animals (5/sex/group) were assigned to one of 4 groups: Group 1 – placebo (10 mL distilled water/kg body weight/day); Group 2 – 40 mg lactium™/kg body weight/day; Group 3 – 200 mg lactium™/kg body weight/day; Group 4 – 1,000 mg lactium™/kg body weight/day. The study evaluated body weights on Days 1, 8, 15, 22, and 28, food consumption over 48-hour periods, and general clinical signs daily. All animals were sacrificed and necropsied on Day 29.

There were no mortalities, clinical signs, body weight gain or food consumption treatment related changes. Minor changes in hematology parameters included slight decreases in hematocrit (in males and females receiving 200 mg/kg/day, and in females receiving 1000 mg/kg/day), Mean Corpuscular Volume (in males receiving 200 mg/kg/day), and leucocyte count and lymphocytes (in males receiving 40 or 1000 mg/kg/day). These variations, although reaching statistical significance ($p<0.05$) were not considered to be biologically significant since the differences were small, there was no dose response relationship, and the majority of treated animals had individual values near or within the range of the controls. Prothrombin time was increased in males and females receiving 1000 mg/kg/day; however, this response was also not considered biologically relevant since (except for one male animal in group 4) individual animals

in the treatment group had prothrombin times near or within the range of animals in the control group. Small but statistically significant increases in serum aspartate amino transferase (AST) and alanine amino transferase (ALT) were observed in males treated with mid and high doses, compared to controls. As these findings were not dose related, were not associated with changes in liver weights or histopathology and were near or within the limits of control values, they were not considered to be biologically relevant. No significant differences in any clinical chemistry parameters were observed in treated females compared to controls.

Gross pathology and organ weight examinations were determined in all control and treated animals. Histopathology examinations were conducted in animals from the control and high dose group. No treatment-related gross pathology, histopathology or organ weight changes were observed. Minor hepatic changes (microvacuolations of hepatocytes; small diffuse granuloma around centrilobular veins) observed in high-dose animals were not considered to be toxicologically significant since they were also observed in controls with the same severity and frequency and are considered common to this animal species. Microvacuolation of the hepatocytes is also partly due to fasting before sacrifice (glycogen reducing). Based on the lack of significant toxic effects, the no-observed-adverse-effect levels (NOAEL) was determined to be 1000 mg/kg/day, the highest dose tested.

(iii) Genotoxicity

The mutagenic potential of lactium™ was examined in the *in vitro* mammalian cell gene mutation assay in L5178Y mouse lymphoma cells (Forichon *et al.* 2001). *(appendix 6)*. The study was also reportedly conducted in accordance with GLP, and in compliance with the relevant guidance from the ICH, and following a protocol adapted from guideline 476 by the OECD, FDA Redbook II and EPA part 798, Sec. 798-5300. Cells were treated for the short exposure time (4-hour treatment) in the presence and absence of metabolic activation; the long exposure time (24-hour treatment) was examined in the absence of metabolic activation. Concentration levels of 1.7, 5.4, 17, 52, 164, 512, 1,600, and 5,000 µg/mL were examined and all treatments were conducted in duplicate. The study included negative (water vehicle) and positive controls; methylmethanesulfonate (4 and 7.5 µg/mL) and cyclophosphamide (2.5 and 5 µg/mL) were used in the absence and presence of metabolic activation, respectively.

No precipitate or cytotoxicity was observed in any of the treatments and all cultures were analyzed for mutation frequencies. No statistically or biologically significant increases in mutation frequency compared to the negative control were observed at any lactium™ concentration. It was concluded that, under the conditions of this assay and according to the criteria of the protocol, lactium™ was not mutagenic when tested up to 5,000 µg/mL using either long or short periods of treatment.

The mutagenic potential of lactium™ at concentration levels ranging from 50 to 5,000 µg/plate was also examined in the bacterial reverse mutation assay in four histidine-dependant strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one tryptophan-dependant strain of *Escherichia coli* (WP2 *uvrA* pKM101) in the presence and absence of metabolic activation (Forichon *et al.*, 2000). *(appendix 7)*. The protocol was adapted from OECD guideline 471, FDA Redbook II, EEC guideline 92/69 (Annex V – method B14, EPA part 798, Sec. 798.5265, MAFF guideline 4200 and MHW notification no. 24. However, the relevance of this assay is questionable given the presence of tryptophan and histidine in the test article (1.2 and 2.25%, respectively), which can interfere with the measurement of mutant frequency. No precipitate or cytotoxicity was observed in any of the treatments. The number of revertant colonies significantly increased with dose in the strains WP2 *uvrA* pKM101 and TA100 using the preincubation method with metabolic activation, only. Due to the presence of tryptophan and histidine in the test article, which can confound the effects noted, it was not possible to conclude if lactium™ exhibited any mutagenic potential. It was therefore concluded that the Ames test was not relevant in the evaluation of the mutagenic potential of lactium™.

Overall, study results indicate that lactium™ lacked mutagenic potential in the *in vitro* mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. The lack of mutagenic potential is supported by the fact that consumption of bovine milk which results in some exposure to the components of lactium™ is known to occur routinely throughout life.

(iv) Developmental Toxicology

The effect of lactium™ on fertility and developmental parameters was examined in pregnant Wistar rats (8/group) assigned to 1 of 5 oral treatment groups: Group 1, placebo (150 mg skimmed milk powder/kg body weight/day); Group 2, 150 mg lactium™/kg body weight/day during the gestation period; or Groups 3 to 5, 150 mg lactium™/kg body weight/day during Week 1, 2, or 3 of gestation only, respectively (Messaoudi *et al.* 2001). *(appendix 8)*. The vehicle used for placebo and test article was 0.5% methylcellulose; in the periods in which animals were not treated, they were administered 5 mL vehicle/kg body weight. Duration of gestation, litter size, litter sex ratio, maternal behaviour (nest construction quality, material used), and care of offspring were assessed in dams. After determination of litter size, litters were culled to 8 rats (sex ratio close to one) for physical and neuromotor testing. After weaning, 2 animals/sex were randomly selected for behavioural and cognitive testing. Offspring were assessed for physical development (weight gain, age of incisors piercing and eyes opening), neuromotor development between birth and weaning (Eversion test, Grip test, Righting reflex test, Wire-hanging test, Locomotion coordination test), and behavioural and cognitive development (Open Field test, Morris water maze test, discriminating learning test, such as ALSAT).

The pregnancy times of the females were statistically equivalent in the various treatment groups. Litter size, litter sex ratio, maternal behaviour and care of offspring of females treated with lactium™ were all comparable to those of control females. There were no significant differences in physical or neuromotor development, or in behavioural and cognitive development between young born from treated and control females. Given that there were no significant differences between treatment groups (in either dams or offspring) in any of the endpoints examined, it was concluded that under the conditions of this study, lactium™ was not associated with any adverse maternal effects on gestation length, maternal behaviour, care of offspring or on physical, leucomotor, behavioural, or cognitive development in the offspring.

A teratogenicity study was conducted on lactium™ in Wistar rats (Plenat, 2001a) *(appendix 9)*. to evaluate any effects of lactium™ given orally to rats throughout pregnancy on the young (F2) from the litters (F1) of the treated rats. Dams (4/group) were treated orally with 150 mg lactium™ or placebo (skimmed milk)/kg body weight/day during the gestation period. A 3-week old male and female offspring were randomly selected from every litter and examined for general external, internal visceral, and skeletal malformations; histological examinations were also performed. There were no micro- or macroscopic changes in offspring that were associated with treatment with lactium™. It was concluded that under the conditions of this study, lactium™ is not teratogenic.

A similar study by the same author found that an oral dose of 150 mg lactium™ /kg body weight/day to female Wistar rats throughout pregnancy did not induce general external or internal visceral malformations in the F2 offspring. No histological changes were noted following examination (Plenat, 2001b). *(appendix 10)*.

The no observable adverse effect level (NOAEL) for developmental effects determined for the above studies was 150 mg/kg body weight/day, the highest dose tested.

(v) Special Studies

The following special studies were designed and conducted based upon the fact that a number of physiological benefits have been attributed to the consumption of milk over the years. These have included gastroprotective effects in rat stress-induced ulcer models and numerous anecdotal reports of sleep improvement and calming (Miclo *et al.,* 2001). *(appendix 11)*. Furthermore, milk proteins are the only ones synthesized by mammals in order to feed their progeny. The effects of caseins were considered since it is known that their enzymatic hydrolysis produces peptides with various biological activities including, amongst others, opiod and opiod-antagonist peptides, angiotensine-converting-enzyme inhibitors, immunostimulating peptides and antibacterial peptides (Miclo *et al.,* 2001). The physiological effects of the

peptides from milk proteins were therefore examined to determine potential sedative and calming properties in the following animal and human models.

The addictive effect of lactium™ was examined in male Wistar rats (n=40) using the Conditioned Place Preference test (Messaoudi *et al.*, 1999a). *(appendix 12)*. A dose of 1 mg decapeptide α_{s1} casein (f91-100)/kg dissolved in 0.9% NaCl was administered intraperitoneally on Days 4, 6, 8, and 10; vehicle (saline) was injected on the alternate days. The control rats were treated intraperitoneally with 3 mg Diazepam/kg according to the same design plan. Diazepam did show an addictive effect, however α_{s1} casein (f91-100) did not show any addictive effects, since it did not induce conditioned place preference in the male Wistar rat.

A double-blind test was used to examine the possible amnesic effect of α_{s1} casein (f91-100) administration *vs.* Diazepam (2 mg/kg i.p.) and saline on social memory, as assessed by change in the duration of investigations of the ano-genital areas of a juvenile by the treated adult rat on the second exposure compared with the first (Messaoudi *et al.*, 1999b). Each male Wistar rat (n=48) was exposed to a juvenile for a 5-minute period and then received an intraperitoneal dose of 0.4 or 0.8 mg α_{s1} casein (f91-100)/kg body weight dissolved in 0.9% NaCl. Thirty minutes after dosing, treated rats were exposed to the same juvenile for a second 5-minute period. Investigations of the adult rats were defined as direct contact (sniffing, nosing) with the ano-genital areas of the juvenile. Experimenters who recorded the behaviours of the rats were unaware of the administered products.

Within the first minute of the 5-minute period, rats treated with saline or decapeptide had significantly decreased duration of investigations on the second exposure compared with the first; rats treated with Diazepam spent equal time investigating the juvenile within the first minute of the first and second exposures. Over the 5-minute period, rats treated with saline or lactium™ also had significantly decreased duration of investigations on the second exposure compared with the first; rats treated with Diazepam spent significantly increased time investigating the juvenile over the 5-minute period of the second exposure compared with the first exposure.

Behavioural effects due to the bioactive decapeptide $_{s1}$ casein (f91-100) administration *vs.* saline were observed in male Wistar rats in 2 separate studies: in the morning and afternoon (Messaoudi *et al.*, 1999a,b) *(Appendix 13)* ; assessed by the Irwin test. In each study, the decapeptide was administered intraperitoneally at doses of 0.5, 1.5, and 4.5 mg/kg, 30 minutes before observation. Behaviour (spontaneous activity, motor affected response, sensorimotor response), neurology (posture, muscle tone, equilibrium and gait, CNS excitiment), autonomic nervous stimulation (eyes, secretions and excretions) and mortality were assessed. The anti-stress characteristics, observed in the morning study were decreased startle reaction in the high-dose group, decreased reaction to touch escape in the low-dose group, and decreased

reaction to tail pinch at all treatment doses. In the high-dose group, lactium™ induced irritability at 30, 60, and 120 minutes after treatment administration. Characteristics of anti-stress observed in the afternoon study were decreased reaction to startle in the low- and mid-dose groups, and decreased reaction to touch escape at all treatment doses. Doses of 0.5 and 4.5 mg lactium™/kg induced body tone (*i.e.* resistance to compression between to fingers) at 120 minutes after treatment administration. It was concluded the safety profile was satisfactory in both behavioural effects studies.

There was no reported tolerance to the anxiolytic effect of the decapeptide α_{s1} casein (f91-100) in male Wistar rats when administered a 12 mg/kg dose intraperitoneally twice daily for 4 days, followed by an intraperitoneal dose of 6 mg/kg on Day 5 (Messaoudi *et al.*, 1998c). *(Appendix 14)*. Diazepam (2 mg/kg i.p.) was used as a reference, and dosed according to the same design plan. Tolerance was assessed by the Conditioned Defensive Burying Test using time spent burying a probe, number of stretchings towards the probe, number of approaches towards the probe, and number of retreats away from the probe as variables to score total stress of each rat.

Clinical Studies

Two clinical studies on lactium™ were conducted in healthy, normotensive subjects.

A double blind, placebo controlled, randomized study (Bresson *et al.*, 2000) (*Appendix 15*) examined the effect of lactium™ administration *vs.* skimmed milk placebo on mental and physical stress, as assessed by the Stroop and Cold Pressor tests, respectively. Male Caucasian subjects (n=42) received two capsules of lactium™ (200 mg) or placebo (200 mg) in the morning and evening of Day 1 of the study, and again on the morning of Day 2. Assessment on Day 2 involved the Stroop test preceded and followed by a 5- and 30-minute relaxation period, respectively; the Cold Pressor test followed by a 5-minute relaxation period. Assessment of systolic and diastolic blood pressures, heart rate, at rest and during stress testing, and plasma ACTH and cortisol levels at rest and after both stress tests were completed. Values pre- and post-stress testing were compared using 2-tailed t-tests. Adverse effect monitoring was not reported and no subjects were reported to discontinue the study.

Twenty subjects in the placebo group and 19 subjects in the treated group were assessed during the Stroop test. At the 0.0001% level of significance, systolic and diastolic blood pressures in lactium™-treated subjects when measured during the Stroop test were significantly increased (14.47 and 15.04% of baseline, respectively) compared to values measured before the Stroop test. Increases in systolic and diastolic blood pressures during Stroop testing in the placebo group (21.27 and 21.14%, respectively) were also significantly different than those obtained pre-Stroop testing. Heart rate increased significantly, 13.70 and 14.20% in the placebo and lactium™ groups, respectively, during Stroop testing compared to rest values.

Both systolic and diastolic blood pressures deviation percentages in the control group were significantly higher than that of the treatment group during Stroop testing, however no significant difference in heart rate was noted.

Sixteen subjects in the placebo group and 16 subjects in the treated group were assessed during the Cold Pressor test. At the 0.0001% level of significance, systolic and diastolic blood pressures in lactium™-treated subjects when measured during the Cold Pressor test were significantly increased (23.20 and 23.40% of baseline, respectively) compared to values measured before the Cold Pressor test. Increases in systolic and diastolic blood pressures during Cold Pressor testing in the placebo group (27.60 and 31.40% of baseline, respectively) were also significantly different than those obtained pre-Cold Pressor testing. Compared to pre-test resting values, the heart rate for the placebo group increased significantly (5.04%) during Cold Pressor testing but no significant difference was observed in lactium™-treated subjects. No significant differences in systolic blood pressure or heart rate deviation percentages were noted between groups during testing. Diastolic blood pressure deviation percentage tended to increase in the control group compared to the treatment group during Cold Pressor testing, however not significantly.

Twenty-one subjects in the placebo group and 21 subjects in the treated group were assessed for cortisol and plasma ACTH content before and after all the stress tests (pre- and post-testing). Plasma ACTH levels (adrenocorticotropic hormone) tended to increase in placebo group (mean values 26.86 and 50.16 pg/mL pre- and post-testing, respectively) and to decrease in the treated group (mean values 35.59 and 28.11 pg/mL pre- and post-testing, respectively) over the testing session, however these changes were not significant at the 0.09% level of significance. No significant difference in plasma ACTH levels deviation percentages between the placebo and treatment groups were reported. In contrast, plasma cortisol content in the placebo group remains statistically stable between pre- and post-testing (mean values 17.17 and 15.60 µg/dL pre- and post-testing, respectively), while the plasma cortisol content of the treated group is significantly reduced between pre- an post-testing at the 0.005% level of significance (mean values 19.70 and 15.50 µg/dL pre- and post-testing, respectively). No significant differences in plasma cortisol content deviation percentages between the placebo and treatment groups were reported.

The study concluded that in the Stroop test, a situation of moderate stress, lactium™ exhibited anxiolytic-like effects based on systolic and diastolic blood pressures; in the Cold Pressor test, a situation of stress associated with pain, lactium™ exhibited anxiolytic-like effects based on heart rate. lactium™ significantly reduced plasma cortisol content within treated subjects during the stress tests.

A 30-day double blind placebo controlled randomized study examined the effects of 150 mg lactium™ *vs.* 150 mg of skimmed milk placebo in males and females (Bourdon *et al.*, 2003) *(Appendix 16)*. Subjects ingested an oral dose of 150 mg lactium™ each evening from Days 1 to 31, followed by a 12-day washout period post dosing. Subjects underwent a mental stress test pre-dosing (Day 0, baseline), on Days 11, 31, and 43. Each testing session consisted of the Stroop test preceded and followed by a 5-minute relaxation period (rest and recovery periods, respectively). Reactivity (measure of the difference between end of the recovery relaxation period and the beginning of the stress test) of mean blood pressure, salivary cortisol level, and arousal (assessed using the Thayer Activation-Deactivation Adjective Checklist) to the Stroop test were assessed. Changes from basal readings in ambulatory heart rate, systolic and diastolic blood pressures, night urinary cortisol levels, and anxiety (assessed using the Spielberger's State Anxiety Inventory) were also assessed. Endpoints were compared using ANCOVA and Student t-tests. Adverse effect monitoring was conducted at the end of the 30-day treatment and 12-day recovery periods.

Fifty-two subjects (n=27 treated, n=25 placebo) completed the study. Of the reactivity measures, mean blood pressure stress reactivity was lower at Days 11 and 31 in the treated group; salivary cortisol levels were not significantly increased after stress testing; and response to the Thayer Activation questionnaire was not affected. Of the endpoints examined for changes from baseline values, there was no significant effect on ambulatory cardiovascular parameters or urinary cortisol levels, and at Days 11 and 31, there were no significant changes in anxiety or arousal in either treated or placebo groups. There were no adverse effects observed during treatment or recovery periods, nor were any study dropouts reported.

Subjects at D0 were subsequently classified as low- (n=37) or high-stress (n=14) responders on the basis of the systolic blood pressure reactivity (measure of the difference between end of the recovery relaxation period and the beginning of the stress test) and the trait-anxiety (anxiety scale trait) on D0 according to the k-mean method (MacQueen, 1967), after data standardisation. There was no treatment effect on mean blood pressure reactivity for low responders, although heart rate reactivity tended to increase in treated subjects at Days 11, 31 and 43. In contrast, mean blood pressure reactivity was significantly reduced in treated high stress responders at Days 11, 31, and 43. No adverse health effects were reported following the daily consumtion of 150 mg of lactium™ for a period of 30 days.

The study concluded that blood pressure reactivity to mental stress was reduced by daily ingestion of 150 mg lactium™ for 30 days, and that this effect was particularly significant in high stress response subjects.

Dietary Exposure to Hydrolysed Casein

(i) The Composition of lactium™

During the manufacture of lactium™, α_{s1} casein is separated and subsequently hydrolysed by porcine trypsin. The degree of hydrolysis is less than 20%. Thus, the resulting lactium™ product is a casein preparation of polypeptides, oligopeptides, and amino acids. The minimum content of the bioactive decapeptide, α_{s1} casein (f91-100) (molecular mass = 1266.1 Da), is 1.8%. Since the origin of lactium™ is bovine skimmed milk, some exposure to the components of lactium™ would be expected to occur normally in the diet. Also, since some infant formulas are based on cow milk extracts, infants also may be exposed to hydrolysed casein, which is the main component of lactium™. Using this information, the normal dietary exposure to hydrolysed casein from infant formula and bovine milk was evaluated.

(ii) Dietary Exposure of Infants to Hydrolysed α-$_{s1}$ Casein from Infant Formula

A leading manufacturer of infant formula (Mead Johnson Nutritionals) produces a casein infant formula, hydrolysed by a mixture of porcine pancreatic enzymes (Enfalac Nutramigen). Infants that have demonstrated intolerance to regular formulas typically consume this formula. The macronutrient composition of Enfalac Nutramigen is depicted in Table 1, and its percent peptide composition is summarized in Table 2.

Table 1 Macronutrient Composition of Enfalac Nutramigen, a Hydrolysed Casein Infant Formula (Mead Johnson Nutritionals)

	Percent Energy	g/L
Protein	11	68.9
Carbohydrate	44	37.4
Fat	45	18.6

Data obtained from a personal communication with Mead Johnson Nutritionals.

Table 2 Percent Peptide Composition of Enfalac Nutramigen

Peptide Mass (Dal)	Percent, by Weight
<500	60
500 – 999	35
1,000 – 2,000	4
>2,000	Trace (<1)

Data obtained from a personal communication with Mead Johnson Nutritionals.

The dietary exposure of infants to hydrolysed bovine casein is depicted in Table 3. Infants aged one to 6 months exclusively consuming a hydrolysed casein formula are exposed to approximately 4.3 to 5.6 g/day hydrolysed α_{s1} casein. This is far greater than the intended dose of lactium™ in dietary supplements (150 to 400 mg/day). Since, in lactium™, the active decapeptide, α_{s1} casein (f91-100), is present at approximately 1.8%, a dosage of 150 to 400 mg/d lactium™ would correspond to a daily α_{s1} casein (f91-100) intake of 2.7 to 7.2 mg/day. The precise intake of α_{s1} casein (f91-100) from hydrolysed α_{s1} casein in infant formula, if any, is not known; however, the intake of hydrolysed α_{s1} casein peptides with a molecular mass in the range of α_{s1} casein (f91-100) is approximately 172 to 224 mg/d. Thus, the intake of α_{s1} casein (f91-100) from hydrolysed casein infant formula could be comparable to that provided by the intended dose of lactium™.

Table 3 The Intake of Bovine Hydrolysed Casein from Enfalac Nutramigen

Age (mo)	Formula Intake (mL/d) [A]	Total Hydrolysed Casein Intake (g/d) [B]	Total Hydrolysed α_{s1} Casein Intake (g/d) [C]	Intake of α_{s1} Casein Peptides 1,000 to 2,000 Da (mg/d) [D]
1	673	12.5	4.3	172
3	782	14.5	4.9	196
6	896	16.6	5.6	224

[A] Estimated from the mean daily breast milk intakes for infants aged one to six months (U.S. EPA, 2002). A density of 1.0 g/mL was assumed for milk. Infants were assumed to be exclusively formula-fed. *(Appendix 17).*
[B] Estimated by multiplying the formula intakes by 18.6 g/L.
[C] Estimated by multiplying the total hydrolysed casein intake by 34%, since 34% of casein molecules are α_{s1} (Fennema, 1996).
[D] Since α-casozepine has a molecular mass of 1266.1 Da, the exposure of infants to peptides from the α-$_{s1}$ caseinate fraction with a molecular mass in the range of 1,000 to 2,000 Da was calculated by multiplying the total hydrolysed α-$_{s1}$ casein intake by 4%.

(iii) Dietary Exposure to Hydrolysed α_{s1} Casein from Bovine Milk

The macronutrient composition of bovine milk is depicted in Table 4. The protein content of milk is about 34 g/L, regardless of the fat content. Casein proteins represent 80% of milk proteins, and 34% of total caseins are α_{s1} caseins (Fennema, 1996). *(Appendix 18).* Using this data, it is possible to estimate the daily intake of hydrolysed α_{s1} casein from milk (Table 5).

Table 4 Macronutrient Composition of Bovine Milk							
		Protein		Carbohydrate		Fat	
		% Energy	g/L	% Energy	g/L	% Energy	g/L
Milk	Skimmed	38.7	34.2	58.7	51.8	2.6	1.0
	Semi-skimmed	27.7	34.1	42.0	51.7	30.3	16.5
	Whole	19.1	33.0	28.6	49.5	52.3	40.2

Calculated from Holland *et al.*, 1996 *(Appendix 19)*.

During normal digestion, proteins are metabolized completely by three proteolytic enzymes, namely pepsin, trypsin, and chymotrypsin. Thus, bovine milk proteins would be completely hydrolysed, and the level of exposure to hydrolysed bovine α_{s1} casein would be similar to the amount of casein ingested.

The intake of fluid milk in children and adults also results in dietary exposure to casein although typically at lower levels than in infants. Exposure to α_{s1} casein and α_{s1} casein hydrolysates is greatest in children 5 years of age and younger (4.0 g/d) and lowest in adults 20 years of age and older (1.7 g/d). In both these cases, the levels are greater than the intended therapeutic dose of lactium™ (150 to 400 mg/day). Since the bioactive decapeptide, α_{s1} casein (f91-100) (molecular mass 1266.1 Da), represents about 1.8% of the intended lactium™ dose, an intake of 150 to 400 mg/day lactium™ corresponds to a α_{s1} casein (f91-100) intake of 2.7 to 7.2 mg/day. Assuming that, similar to the hydrolysed casein infant formula, 4% of peptides have a molecular mass in the range of 1,000 to 2,000 Da, the intake of α_{s1} casein peptides 1,000 to 2,000 Da would be 68 to 160 mg/day. Again, the precise intake of α_{s1} casein (f91-100) from hydrolysed α_{s1} casein from bovine milk is not known, however some exposure to α-casozepine is considered likely.

Table 5 Dietary Exposure to Hydrolysed α_{s1} Casein from Bovine Milk					
Age (yr)	Fluid Milk Intake (mL/d) [A]	Protein Intake (g/d) [B]	Casein Intake (g/d) [C]	α_{s1} Casein Intake (g/d) [D]	Intake of α_{s1} Casein Peptides 1,000 to 2,000 Da (mg/d) [E]
≤5	433	14.7	11.8	4.0	160
6-11	369	12.5	10.0	3.4	136
12-19	304	10.3	8.2	2.8	112
≥20	179	6.1	4.9	1.7	68

[A] Based on the mean USDA CSFII 1994 and 1995 data for dairy products intake per individual in a day, by sex and age (U.S. EPA, 1997). *(Appendix 20)*. A density of 1.0 g/mL was assumed for milk.
[B] Determined by multiplying the total fluid milk intake levels by 34 g/L.
[C] Determined by multiplying the total protein intake from fluid milk by 80%.
[D] Determined by multiplying the total casein intake from fluid milk by 34%.
[E] Determined by multiplying the total α_{s1} casein intake from fluid milk by 4.0%. In performing this calculation, it was assumed that: (1) All the α_{s1} casein would be hydrolysed; and (2) Since protein digestion would be facilitated by a

mixture of proteolytic enzymes (not just trypsin as in lactium™), the level of peptides with a molecular mass in the range of 1,000 to 2,000 Da would be 4.0% (i.e. similar to that found in the hydrolysed casein infant formula [Enfalac Nutramigen]).

Generally Recognized as Safe Status of Peptones

Peptones are affirmed as Generally Recognized as Safe (GRAS) by the FDA (21CFR Sec. 184.1553). Chemically, as demonstrated in the attached identity, lactium™ is a peptone which by definition is a variable mixture of polypeptides, oligopeptides, and amino acids that are produced by partial hydrolysis of casein, animal tissue, soy protein isolate, gelatin, defatted fatty tissue, egg albumin, or lactalbumin (whey protein). Peptones are produced from these proteins using proteolytic enzymes that either are considered to be GRAS or are regulated as food additives. The enzyme used to produce lactium™, trypsin EC3.4.21.4. is affirmed GRAS under 21CFR Sec. 184.1914.

In agreement with 21CFR Sec. 184.1(b)(1), peptones can be used in food as nutrient supplements, as processing aids, and as surface-active agents with no limitation other than current good manufacturing practice (GMP). Nutrient supplements are defined as substances which are necessary for the body's nutritional and metabolic processes 21CFR Sec. 170.3(o)(20).

The GRAS affirmation of peptones as nutrient supplements provides additional support that lactium™ lacks toxic potential.

ASSESSMENT OF SAFETY

The safety of lactium™ is supported by existing dietary exposures of the components of the NDI from consumption of milk, and by animal toxicology studies and human clinical trials. Furthermore, lactium™ meets the definition of a peptone, which is GRAS under 21CFR Sec. 184.1553 for use in food with no limitation other than GMP.

lactium™ contains hydrolysed α_{s1} casein as polypeptides, oligopeptides and amino acids. Since it is obtained from bovine skimmed milk, humans are already likely to be exposed to the components of lactium™ through the diet. Exposures to hydrolysed α_{s1} casein (and possibly α_{s1} casein (f91-100)) from use of specialized infant formulas are actually considerably greater than from lactium™.

The maximum recommended intake of lactium™ of 400 mg/day (or approximately 6.7 mg/kg body weight/day assuming a body weight of 60 kg) is about 150 times lower than the NOAEL determined from the subchronic rats study. This margin of safety is sufficient to support the use

of lactium™ at the maximum dose of 400 mg/day. In addition, the results of an *in vitro* mammalian cell gene mutation assay indicated that lactium™ lacks mutagenic potential and lactium™ was not found to cause any adverse effects in three developmental toxicology studies. In two clinical trials, no adverse effects were reported among subjects administered either 800 mg lactium™ on day 1 and 400mg on day 2 or 150 mg lactium™/day for 30 days, respectively.

Based on the available data, we have concluded that the use of lactium™ as a New Dietary Ingredient, at the recommended intake of between 150 and 400 mg/day, is safe.

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